Validation of a disease model in Japanese quail *(Coturnix coturnix japonica)* with the use of *Escherichia coli* serogroup O2 isolated from a turkey

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Abstract

This study established a disease model and protocol for bacterial challenge with *Escherichia coli* serogroup O2 strain EC317 in Japanese quail. Five groups of 10 birds each were injected subcutaneously in the breast with 200 μL of a brain–heart infusion (BHI) culture containing 1×10^8, 1×10^7, 1×10^6, 1×10^5, or 1×10^4 colony-forming units/mL of the test organism, which had been isolated from a turkey with cellulitis and septicemia. Birds in a 6th group were controls that received sterile BHI alone. Localized lesions of cellulitis developed in all of the birds that received *E. coli*. The morbidity and mortality rates were highest (100%) in the birds receiving the highest dose of *E. coli* and decreased linearly with decreasing dose ($P < 0.05$). Severity of disease, including lesions of pericarditis and perihepatitis, was also directly proportional to the dose of *E. coli*. These findings indicate that this disease challenge protocol can be used to study disease resistance and immunologic consequences of contaminant exposure or other stressors in birds.

Résumé

Cette étude a permis de développer un modèle de maladie et un protocole pour l'inoculation défi avec Escherichia coli *sérogroupe O2 souche EC317 chez la caille japonaise. Cinq groupes de 10 oiseaux chacun ont été injectés par voie sous-cutanée au niveau de la poitrine avec 200 μL de culture en bouillon cœur-cervelle (BHI) contenant 1×10^8, 1×10^7, 1×10^6, 1×10^5 ou 1×10^4 unités formatrices de colonie/mL (UFC/mL) du micro-organisme test, isolé d'un cas de cellulite et de septicémie chez une dinde. Des oiseaux dans un 6^e groupe ont servi de témoins et ont reçu uniquement du BHI stérile. Des lésions localisées de cellulite se sont développées chez tous les oiseaux ayant reçu du* E. coli. *Les taux de morbidité et de mortalité étaient plus élevés (100 %) chez les oiseaux recevant la dose la plus élevée de* E. coli *et ont diminué de façon linéaire avec des doses décroissantes* ($P < 0,05$). *La sévérité de la maladie, incluant les lésions de péricardite et de périhépatite, était également directement proportionnelle à la dose administrée de* E. coli. *Ces données indiquent que ce protocole d'inoculation défi peut être utilisé pour étudier la résistance à la maladie et les conséquences immunologiques d'exposition à des contaminants ou à d'autres facteurs de stress chez les oiseaux.*

(Traduit par Docteur Serge Messier)

Introduction

Immunotoxicity, the immunomodulatory effects of chemicals on the immune system (1), is used to assess contaminant-induced adverse effects on animals and humans. Birds are commonly used as indicators for assessing ecosystem health and sustainability related to persistent or current use of low-residue environmental contaminants (2). To date, immunotoxicity studies investigating the effects of chemicals on avian species have been based on a chicken model (3). In recent experimental studies Japanese quail *(Coturnix coturnix japonica)* have been used to investigate the immunotoxicologic effects of environmental toxicants (4,5). Quail are preferred over domestic poultry *(Gallus gallus)* as avian models because of their similarity to wild species and because they have not been as intensively selected and bred by the poultry industry for traits of economic importance, such as eggs and production. These features, plus their easy availability, make quail an excellent model for investigating toxicologic effects, disease resistance, and immune function in wild birds. There are numerous reports on in vitro or in vivo immune function in birds related to exposure to environmental contaminants (4,5), but none describes the evaluation of functional immunity in the face of concurrent bacterial or viral infection. Hence, the present study was conducted to develop a bacterial challenge model that could be used for meaningful immunotoxicity testing.

Escherichia coli infections in broiler chickens have been associated with a wide range of conditions, including cellulitis, septicemia, airsacculitis, perihepatitis, omphalitis, respiratory tract infections, and gastrointestinal tract infections (5–7). Acute forms of *E. coli* infection produce septicemia, whereas subacute or chronic infections result in pericarditis, perihepatitis, airsacculitis, and arthritis. Similar conditions have been described with *E. coli* infection in farmed quails (8,9).

Because *E. coli* O2 and O78 have been used for disease challenge models in poultry (7,10), we tested the usefulness of an *E. coli* challenge model in Japanese quail to determine the bacterial doses and pathological responses to *E. coli* serogroup O2 exposure. The main purpose of achieving these objectives was to produce validated baseline information on *E. coli* serogroup O2 as a disease challenge organism causing clinical signs and pathological responses in a

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Table I. Clinical and pathological results in groups of Japanese quail (10 per group) injected subcutaneously with 200 μL of brain–heart infusion (BHI) or various doses [colony-forming units (CFU) per milliliter of BHI] of *Escherichia coli* serogroup O2 strain EC317

		Dose of *E. coli* (CFU/mL); % of group affected				
Parameter	Control	1×10^4	1×10^5	1×10^6	1×10^7	1×10^8
Death or illness necessitating euthanasia	0	30	50	60	70	100
Cellulitis	0	100	100	100	100	100
Pericarditis	0	20	30	40	50	40
Airsacculitis	0	10	30	40	40	40
Perihepatitis	0	20	30	40	50	30
Arthritis	0	0	10	0	20	20

predictable percentage of the challenged quail under normal husbandry conditions. Such a model would be valuable for researchers studying toxicologic and immunologic responses in birds.

Materials and methods

Animal source and management

The experiment was conducted with 60 Japanese quail that were obtained from Fircrest Farms, Langley, British Columbia. The 9-week-old quail were randomly allocated to 6 floor pens. Equal numbers of birds ($n = 10$) were randomly allocated to each treatment. The pens were in a room with controlled ventilation and a temperature maintained at 20°C to 21°C. Nonmedicated turkey grower feed and water were provided ad libitum. Body weight data were collected before *E. coli* challenge and at the time of death.

The experimental protocols were approved by the Animal Care Committee, University of Saskatchewan, Saskatoon, Saskatchewan, and all procedures were performed in accordance with the requirements of the Canadian Council on Animal Care (11).

Bacterial culture and challenge

A clinical isolate of *E. coli* serogroup O2 strain EC317 from a turkey with cellulitis, pericarditis, and airsacculitis was obtained from the Vaccine and Infectious Disease Organization at the University of Saskatchewan. The bacteria were grown in brain–heart infusion (BHI) medium after being passaged once on MacConkey agar for 13 h at 37°C. Absorbance was measured with the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA) at a wavelength of 600 nm, and the number of bacteria was counted by a standard plate count method. To correlate absorbance and numbers of *E. coli*, a standard curve was constructed.

After 7 d of acclimation, 5 groups of quail (10 birds per group) were injected subcutaneously in the breast with 200 μL of 1×10^8, 1×10^7, 1×10^6, 1×10^5, or 1×10^4 colony-forming units (CFU) of *E. coli* serogroup O2 strain EC317 per milliliter of BHI per bird. Each bird in the 6th (control) group was injected with 200 μL of BHI. The quail were monitored twice daily for adverse health effects. Blood samples were taken by aseptic technique from all the birds 48 h after *E. coli* injection and when severe signs of distress such as anorexia, recumbency, depression, and poor response to stimulus developed in the 2 wk after challenge.

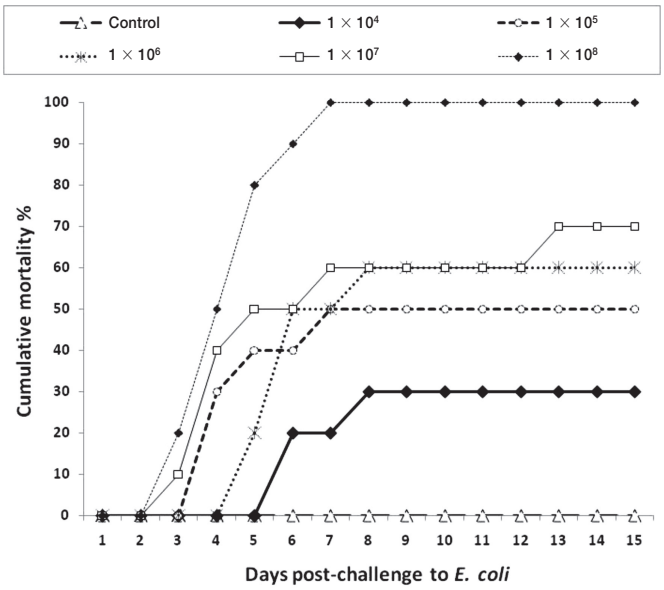

Figure 1. Cumulative rate of death (spontaneous or from euthanasia performed because of severe clinical signs) after subcutaneous injection of Japanese quail with various doses of *Escherichia coli* serogroup O2 strain EC317 (in colony-forming units per milliliter of brain–heart infusion).

On day 21, or earlier if there were severe signs of distress after the bacterial challenge, the quail were euthanized by isoflurane inhalation followed by cervical dislocation. All the birds were examined for gross pathological changes such as cellulitis, pericarditis, perihepatitis, and osteomyelitis, and the presence of these changes was recorded. The skin at the injection site was opened with a sterile scalpel and reflected, and a sterile swab was used to collect samples. In the birds that had died with severe signs of distress, clotted blood samples were taken aseptically from the heart by means of sterile swabs.

Bacterial isolation

Within 24 h the culture swabs were inoculated in 2 mL of BHI broth, cultured for 8 h, and then cultured on MacConkey agar plates

Table II. Change in body mass of the birds over 2 wk after injection of *E. coli* serogroup O2 or BHI alone

	Dose of *E. coli* (CFU/mL)					
Parameter	Control	1×10^4	1×10^5	1×10^6	1×10^7	1×10^8
Average daily weight gain or loss (g/d per bird)	0.95	−0.24	−0.77	−1.09	−1.28	−2.55

for 24 h. Bacteremia was confirmed by incubating 100 μL of blood in the same manner. The birds were classified as septicemic if they had clinical signs of illness (any of anorexia, recumbency, depression, and poor response to stimulus) after the bacterial challenge and were culture-positive.

Phytohemagglutinin (PHA) skin test

Localized blastogenic transformation of peripheral blood lymphocytes is stimulated by PHA, a plant lectin extracted from the kidney bean *(Phaseolus vulgaris)*. The PHA skin test is commonly used in immunotoxicity studies. Because immunologic responses are triggered by exposure to infectious agents, the effects of *E. coli* on T cell immunoreactivity was tested in this study by means of the PHA assay. The skin test was performed 24 h after injection of the highest dose [1×10^8] of *E. coli* or BHI alone, as described by others (12). Briefly, the right wing-web of each bird was injected with 20 μL of the protein form of PHA (PHA-P; Sigma-Aldrich, St. Louis, Missouri, USA), 5 mg/mL, dissolved in phosphate-buffered saline. Wing-web thickness was measured before and 24 h after injection by means of a pressure-sensitive micrometer. Logical constraints allowed PHA testing of only the control and high-dose groups.

Statistical analysis

Regression analysis was performed between dose and incidence of death or severe clinical signs, cellulitis, pericarditis, airsacculitis, perihepatitis, arthritis, bacteremia, and septicemia. Significance was set at $P < 0.05$.

Results

The incidence of death or illness necessitating euthanasia after *E. coli* injection was highest in the birds receiving the highest dose of *E. coli* (1×10^8 CFU/mL) and lowest in the birds receiving the lowest dose (1×10^4 CFU/mL) (Table I). The incidence increased linearly with increasing dose ($P < 0.05$) and peaked on days 3 and 4 after injection (Figure 1). The control group gained an average of 0.95 g/d, whereas in the birds challenged with *E. coli* the body mass decreased linearly with increasing dose of *E. coli* ($P < 0.05$) (Table II).

Cellulitis was observed in all of the birds injected with *E. coli*. Skin lesions of edema and discoloration at the injection site occurred 24 to 36 h after injection. The birds found dead within 48 h after injection had a focal mild inflammatory response over an area of up to 1 × 2 cm, classified as mild cellulitis (Figure 2). The birds that died or were euthanized within 72 h after bacterial challenge had more severe cellulitis, with yellowish brown or brownish red semisolid masses 1 to 5 mm thick in an area ranging from 2 × 3 cm to 3 × 6 cm at the site of injection. The challenge organism was isolated from all the birds with cellulitis.

The proportions of birds with pericarditis and airsacculitis increased linearly with increasing dose of *E. coli* ($P < 0.05$) (Table I). Five birds became lame, and postmortem examination revealed purulent arthritis at the tibiotarsal or tarsometatarsal joint.

Escherichia coli was isolated from the blood of 50% of the birds that received the lowest dose of *E. coli* and 100% of the birds that received the highest dose (Table III). All the birds that died or were euthanized before the end of the study had septicemia. The proportion of birds with septicemia increased ($P < 0.05$) with increasing dose of *E. coli*.

The PHA response was significantly greater ($P < 0.05$) in the control group than in the birds challenged with the highest dose of *E. coli* serogroup O2, the mean increases (and standard deviation) in wing-web thickness being 0.47 ± 0.037 mm and 0.34 ± 0.040 mm, respectively.

Discussion

Our findings show that *E. coli* serogroup O2 can be successfully used as a sound disease challenge model in quail. This protocol demonstrated the capability of *E. coli* serogroup O2 to produce dose-dependent cellulitis, pericarditis, perihepatitis, and septicemia in quail. The characteristics that contribute to the pathogenicity of the *E. coli* O2 strain EC317 have been well-described by others (10,13).

Reports exist describing cellulitis lesions induced by *E. coli* in quail (8,9), but none was based on reproducing the disease in quail. Serogroup O2 EC317 has been used as a model in broiler chickens (7), a species specifically bred for traits of economic importance (high growth rate) and not close to wild species in terms of life history, physiological features, and immune variables. In the chicken model several methods of inducing cellulitis and septicemia, such as plucking the feathers or scratching the skin and then applying *E. coli* (14,15), have been attempted, the most reliable technique being subcutaneous injection, as used in the present study. Subcutaneous inoculation ensures control of the number of bacteria used to produce the desired clinical lesion. Additionally, subcutaneous inoculation is less traumatic and more precise than creating scratch lesions.

This study provides evidence that cellulitis subsequent to *E. coli* colonization in subcutaneous tissue is followed by bacteremia, which, in the heaviest challenges, leads to systemic pathological changes such as pericarditis, perihepatitis, and airsacculitis. To produce cellulitis, bacteremia, and subsequent pathological lesions, *E. coli* needs to overwhelm innate and acquired components of the immune system during the preclinical and clinical phases of the disease.

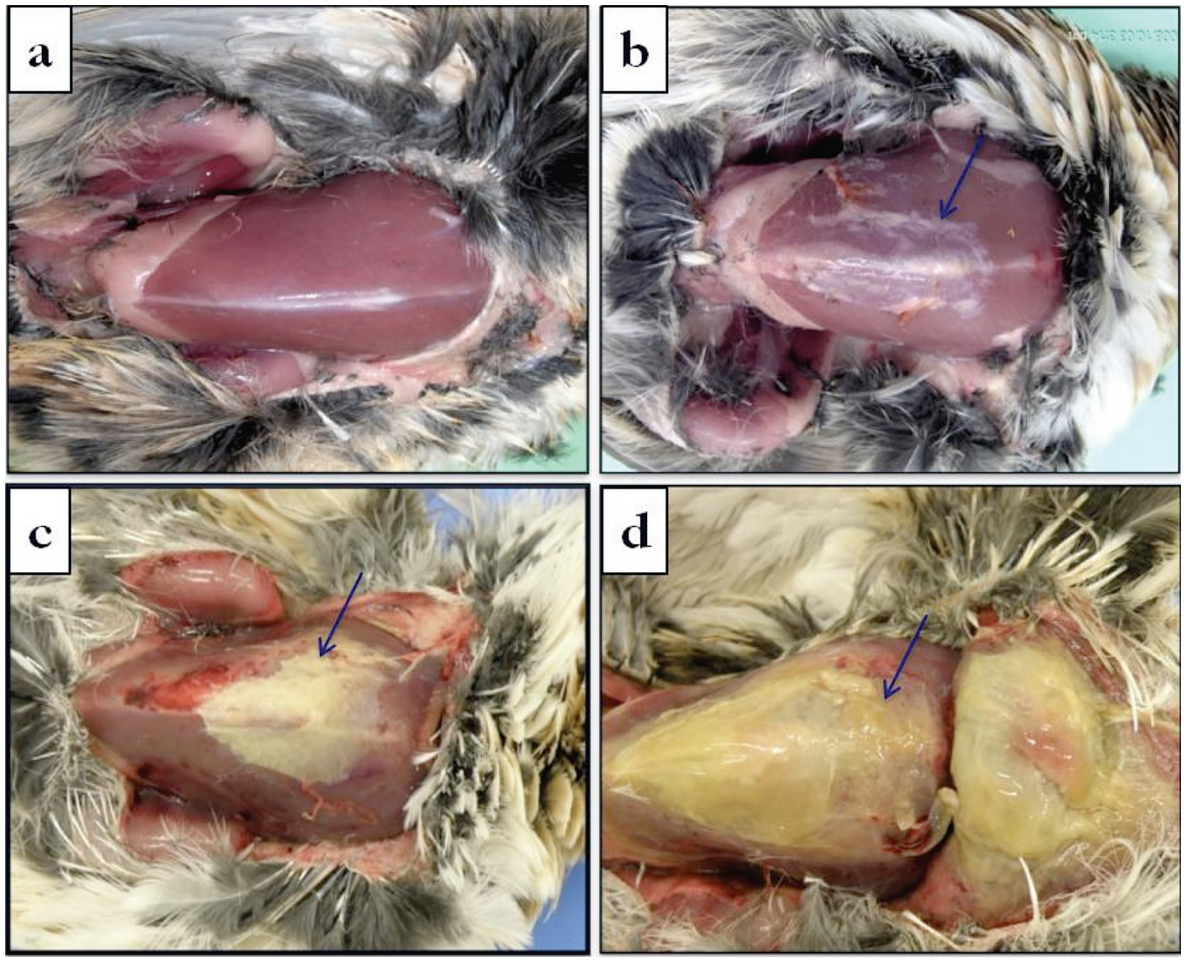

Figure 2. Cellulitis lesions (blue arrows) in quail after subcutaneous injection of *E. coli* serogroup O2. a — no lesions; b — mild cellulitis; c — moderate cellulitis; d — severe cellulitis.

Table III. Bacterial findings in the birds at death or euthanasia

	Dose of *E. coli* (CFU/mL); % of group affected					
Finding	Control	1×10^4	1×10^5	1×10^6	1×10^7	1×10^8
Bacteria isolated from skin lesions	0	100	100	100	100	100
Bacteremia	0	50	60	70	70	100
Septicemia[a]	0	30	50	60	70	100

[a] Birds were so classified if they had clinical signs of illness (any of anorexia, recumbency, depression, and poor response to stimulus) after the bacterial challenge and were culture-positive.

Cellulitis has been described as inflammation of the subcutaneous tissue. In broiler chickens it has been linked with various strains of *E. coli* (15,16). However, not all *E. coli* isolates produce the septicemia experimentally (Nain S. unpublished observations). It is not clear what physiological, biochemical, or genetic factors allow some serogroups to produce systemic infections. The isolate used in this study was from a clinical case of pericarditis and airsacculitis and had the necessary virulence factors to produce the cellulitis and septicemia.

The PHA skin test is a localized measure of the proliferative potential of T lymphocytes, with no systemic effect expected, which provided the rationale for testing only the control group and the group receiving the highest dose. Testing all the groups would have been ideal. The lack of a systemic effect from PHA testing was supported by the linear dose response in terms of both the pathological findings and the morbidity and mortality rates after *E. coli* challenge in spite of the PHA skin test. In rats with peritonitis an integrated test of immune function, the delayed-type hypersensitivity response to keyhole limpet hemocyanin, decreased linearly with increasing amounts of *E. coli* in the gut (17), an observation that supports our findings of a suppressed PHA response in the challenged birds.

Exposure to agricultural and industrial chemicals as well as other environmental stressors can pose threats to the stability of wildlife populations as well as the health of domestic animals. Using a combination of immunotoxicity tests and this validated disease challenge model will allow investigation into the subclinical effects of contaminant exposure in wild birds.

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